

Marker-assisted selection for transfer of submergence tolerance, bacterial blight resistance and yield enhancement in the rice backcross derivatives

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Abstract

Achieving the demand and supply of rice for the increasing global population will be a challenging task beyond 2040. Therefore, the yield potential of popular rice varieties needs to be increased. The production from lowland rice can be enhanced by managing the major biotic and abiotic stresses faced in the ecology. In this work, we have developed the pipelines for gene stacking of submergence tolerance, bacterial blight resistance and yield component QTLs in the popular rice variety 'Swarna' by marker-assisted backcross breeding approach. Genotyping results of 796 BC₁F₁ backcross derived lines showed the presence of *GW2*, *OsSPL14*, *SCM2* yield component QTLs, *Sub1A* for submergence tolerance and bacterial blight resistance genes *xa5* and *Xa21* in the progenies. The four stacked QTLs and two resistance genes were observed in six backcrosses derived progenies only. These progenies will be used in the next generation for further selection and evaluation for enhancing submergence tolerance, yield potential and bacterial blight resistance in Swarna rice variety background.

Keywords: Yield QTL, submergence tolerance, bacterial blight resistance, wealthy farmers panicle, strong culm, backcross breeding.

Abbreviations: QTL_Quantitative trait locus, BBB_acterial blight, MAS_Marker-assisted selection, CTAB_Cetyltrimethylammonium bromide, EDTA_Ethylenediaminetetra acetic acid, TBE_Triss Borate EDTA.

Introduction

Rice is the principal food for the majority of the world's population and is grown under a diverse range of agro-climatic conditions. Rice cultivation is associated with major biotic and abiotic stresses that minimize the productivity of rice from lowland ecosystems. Among abiotic stresses, submergence and water logging are the major constraints for low productivity in the ecology. Submergence due to flash flood is a major problem for rice production in the rainfed lowland rice ecosystem of eastern India, which spreads over around 16 Mha in the country (Pradhan et al., 2016). Bacterial blight (BB) is a common, wide spread critical disease faced by rice plants (*Oryza sativa* L.). It is caused by *Xanthomonas oryzae* pv. *oryzae*. BB is one of the oldest recorded rice disease and it interrupts production in all rice ecosystems.

Our country needs additional rice production of around 2 million tons annually and a total of 135 to 140 million tons by 2030 to continue to remain self-sufficient. This production increase needs to be obtained from less land, less water, less labor and fewer chemicals under the constant threat of new emerging pathogens and pests and possible challenging effects of climate change (Khush, 2005). The global rice production needs to be enhanced by 70% by 2050 to meet the demand of the growing population (FAO, 2009; NRRI, 2013). The current

rice production of India is 110 million tons per annum that need to be enhanced to 150 million tons in the coming 30 years (NRRI, 2013). To achieve this targeted yield level, rice varieties with a yield advantage of about 20% over widely grown varieties must be developed.

The traits influencing yield and tolerance to stresses have been selected by human since time immemorial. Those selections were based on yield component traits. However, many yield QTLs have been identified and also few are cloned during the last few years. Attempts are to be made for the accumulation of these QTLs in a single background for their effects in a positive or negative direction. The important yield component traits such as grain number (*Gn1a*, *Ghd7*, *DEP1* and *WFP*), grain weight (*GS3* and *GW2*), grain size (*GS3* and *GW5*), grain filling (*GIF1*) and panicle number (*DEP1* and *WFP*) may be utilized for enhancing yield potential. Tightly linked and functional markers of these yield related cloned genes may be useful for marker-assisted pyramiding of yield component QTLs. Ashikari et al. (2005) reported the gene stacking of grain number and dwarfing gene combination for enhancement of yield. Swarna is the most popular rice variety in eastern India but the variety is sensitive to submergence and susceptible to

bacterial leaf blight disease. There is a need for submergence tolerant and bacterial blight resistant version of Swarna variety for the eastern region of the country. Also, the yield potential of this variety needs to be enhanced further.

The objective of this study is to stack resistance genes/QTLs in Swarna variety for submergence tolerance, bacterial leaf blight resistance and more yield potential into the popular rice variety through marker-assisted backcross breeding approach.

Results and discussion

Validation of submergence tolerance, bacterial leaf blight resistance and yield component QTLs in the parental lines

The PCR amplification pattern using the genomic DNA of Swarna-Sub1, Swarna MAS, Swarna- Havataki derived lines and susceptible variety Swarna with molecular markers of *Sub1A*, *xa5*, *xa13* and *Xa21* were performed and depicted in the Fig. 1. All the specific lines showed the presence of resistance specific bands indicating the presence of resistance genes *Sub1A*, *xa5*, *xa13*, and *Xa21*, respectively. All donor lines showed the presence of resistance specific bands of 1000 bp, 530 bp and 490 bp, 440 bp and 410 bp indicating the presence of BB resistance genes, *xa5*, *xa13*, *Xa21*, respectively. The genotype Swarna MAS showed the presence and in the case of Swarna-Sub1, Swarna-Havataki derived lines and Swarna there is no band against BB markers. Like that, in Swarna-Sub1 variety, the presence of Sub1A marker was detected and that gene was not in other donor lines viz., Swarna MAS, Swarna-Havataki and Swarna, indicating the absence of these resistance genes.

Generation of F_1 , F_1' and BC_1F_1 seeds

The first hybridization was done between Swarna-Sub1 and Swarna MAS and 205 F_1 seeds were produced. Next season, the true F_1 plant was hybridized with the yield QTLs containing Swarna-Havataki derivative lines and 170 F_1' seeds were obtained. True hybridity was again checked using OsSPL14 gene specific marker and true F_1' plant was hybridized with recipient parent Swarna and 850 BC_1F_1 seeds were produced.

Marker- assisted selection for submergence tolerance, BB resistance and higher yield potential in BC_1F_1 generation

The amplification of the genomic DNA of seven-ninety-six genotypes was performed using gene-based direct markers for strong culm, wealthy farmers' panicle, grain width and submergence tolerance (Table1). Amplification of *SCM2* indicates the presence of strong culm in the amplified genotypes. It is generally considered amplification of *GW2* gene corresponds to the presence of low grain width and higher grain weight (Song et al. 2007). The expression of *OsSPL14* QTL signifies the trait, wealthy farmer's panicle, which elucidates about the overall plant architecture. This study also states that *OsSPL14* gene is also responsible for shoot branching during the vegetative stage. Submergence tolerance of rice a variety is mainly observed by the expression

of *Sub1A* QTL. Direct marker Sub1A203 for submergence tolerance is better than Sub1BC2, this marker is an intergenic marker and is adjacent to Sub1A. Earlier reports suggest that the marker combinations of Sub1A203 + Sub1BC2 could group the genotypes in a more efficient and systematic manner.

There are 42 bacterial blight resistance genes that have been identified from diverse sources (Pradhan et al., 2018). Many of these genes have been tagged by closely linked molecular markers (Gu et al., 2008; Rao et al., 2002; Sonti, 1998; Yoshimura et al., 1995). Among them, 11 are recessive in nature. *xa5* is one of those recessive genes which gives tolerance to bacterial blight along with the dominant gene *Xa21*. In this study, we have taken the above three characteristics and accordingly, we have used molecular markers to screen the presence of submergence tolerance, yield potential and bacterial blight resistance in a number of progenies. All progenies from BC_1F_1 -SM-1 to BC_1F_1 -SM-796 were screened and they exhibited specific bands for various genes as stated below (Fig. 3; Fig. 4). Among these plants, some will be selected for further hybridization for getting the most desirable plant. Grain width and weight may play an important role in increasing grain yield in rice. High grain width increases yield while low grain width increases grain quality of rice. Here, in this present experiment, *GW2* is a functional gene that indicates enhancement in grain width in rice (Song et al., 2007). In this work, out of 796 progenies, 238 progenies harbored *GW2* gene. A strong culm is an important trait of rice plant which provides non-lodging character to the plant. Any plant devoid of strong culm may not be able to bear a heavy panicle load which ultimately may lodge leading to high yield loss. The results from our experiment indicated that two hundred eighty-four genotypes out of seven hundred ninety-six were positive for *SCM2* QTL. Hence, our selection method for strong culm may be effective due to the presence of *SCM2* QTL that imparts sturdy culm to the plant. Previously, Ookawa et al. reported that the presence of *SCM2* provided a strong culm to the rice genotype (Ookawa et al., 2010). Wealthy Farmers Panicle controlled by *OsSPL14* is a useful QTL for panicle trait for increasing yield potential. The direct marker OsSPL14 was employed in the genotyping of the backcross progenies that showed the presence of *OsSPL14* in 274 genotypes out of total of 796 screened genotypes (Fig. 3; Fig. 4). For submergence tolerance, the markers mainly Sub1A203 and Sub1BC2 were used in this study to screen the 796 progenies. The combination of Sub1A203 and BC2Sub1 showed a better resolution as compared to all other combinations. Out of 796 plants, we found Sub1A203 in 163 plants and BC2Sub1 in 365 plants. This combination is considered to be a good combination to screen the submergence tolerance in rice (Pradhan et al., 2016). In this study, we observed the amplification of 143 plants for both the submergence tolerance markers combination. Hence, 143 plants may contain *Sub1* QTL (Fig. 3; Fig. 4). All the desired genes that have been considered in the present work have been cloned and characterized. *Xa21* is a dominant resistant gene that encodes the receptor kinase containing NBS-LRR domains (Song et al., 1995) while *xa5* is a recessive resistance gene and encodes a variant form of transcription factor IIA (Cllalyer and McCouch, 2004). In this present work, we have

Table 1. Molecular markers used for tracking of submergence tolerance, yield component QTLs and bacterial leaf Blight resistance gene.

Sl. No	Characters	Markers	Primer Sequence	Reference
1	Grain width	<i>GW2</i> (F) <i>GW2</i> (R)	5' CCAATAAAGATGTCCATTCTGTTA 3' 5'GCTCTTCTGTAAACACATATTATG 3'	Song et al., 2007
2	Strong Culm	(SCM 2) (F) (SCM 2) (R)	5'ATTGAGATCAATAGGTTGAGTGT3' 5'TGCTATGTATATCCTATCGGTTCC 3'	Ookawa et al., 2010
3	Heavy Panicle & Plant architecture	<i>OsSPL14</i> (F) <i>OsSPL14</i> (R)	5'CAAGGGTCCAAGCAGCGTAA3' 5'TGCACCTCATCAAGTGAGAC3'	Ashikari et al., 2010
4	<i>xa5</i>	<i>xa5S</i> (multiplex) <i>xa5SR/R</i> (multiplex)	5'GTCTGGAATTTGCTCGCGTTCGTGGTAAAGTAGATACCTTATCAA ACTGGAAGCTCGCCATTCAAGTCTTGAG3' 5'TGGTAAAGTAGATACCTTATCAAAGCTCGCCATTCAAGT TCTTGAG TGACTTGGTTCTCCAAGGCTT3'	Pradhan et al., 2015a
5	<i>Xa21</i>	pTA248F pTA248R	5'AGACGCGGAAGGGTGGTCCCGGA3' 5'AGACGCGGTAATCGAAGATGAAA3'	Pradhan et al., 2015a
6	SUB1A	Sub 1 A203 (F) Sub 1 A203 (R)	5' CTT CTT GCT CAA CGA CAA CG 3' 5' AGG CTC CAG ATG TCC ATG TC 3'	Pradhan et al., 2015b
7	Sub1 BC2	Sub 1 BC 2 (F) Sub 1 BC 2 (R)	5' AAAACAATGGTTCCATACGAGAC 3' 5' GCC TAT CAA TGC GTG CTC TT 3'	Pradhan et al., 2015b

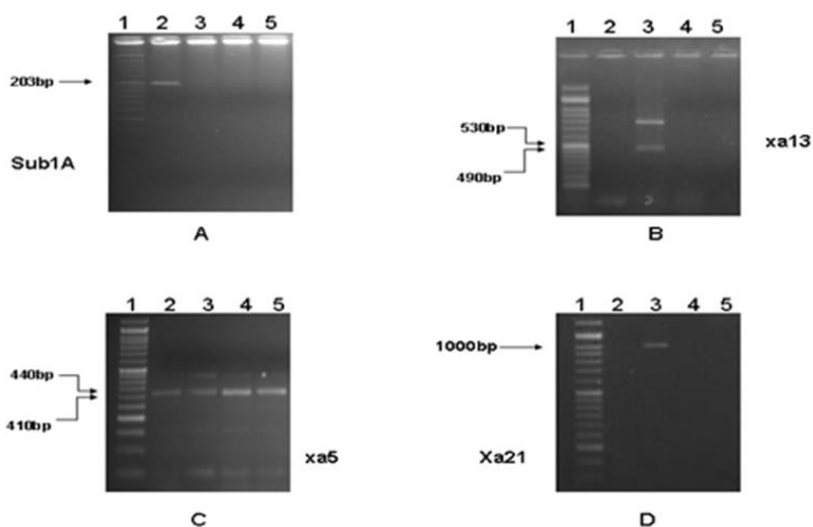


Fig 1. Gel photographs of donor parents (A) presence of expected base pair specific bands (203bp) for Sub1A tolerance QTL in Swarna-Sub1 variety. Lane 1: represents DNA ladders; Lane 2: Swarna-Sub1; Lane 3: Swarna MAS; (B) presence of specific band for BB resistance gene *xa13* (C) specific band for *xa5* and (D) band for *Xa21*.

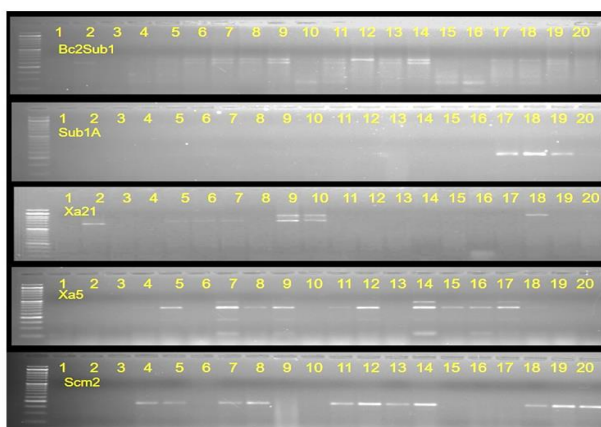


Fig 2. Representative electropherogram of BC₁F₁ derived lines of Swarna varieties using submergence tolerance QTL Sub1A, bacterial blight resistance genes *xa5* and *Xa21* and yield component QTLs *GW2*, *OsSPL14* and *SCM2*. The numbers on the top of the lane indicate the genotype used as in the L:100 bp DNA ladder. Lanes 1-20: Represents backcross progenies from BC₁F₁-SM01 to BC₁F₁-SM20.

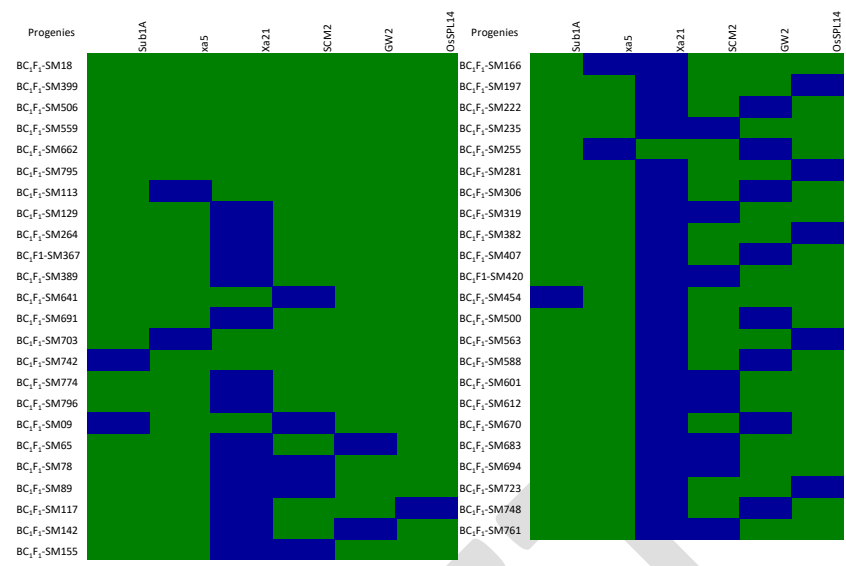


Fig 3. Banding analysis for submergence tolerance, bacterial blight resistance and yield component in BC₁F₁ derived lines for GW2, OsSPL14, SCM2, Sub1A, xa5 and Xa21 genes/QTLs. Blue and green represents absence and presence of a particular gene in a progeny. This figure provides six, five and four genes/QTLs combinations in the genotyping progenies.

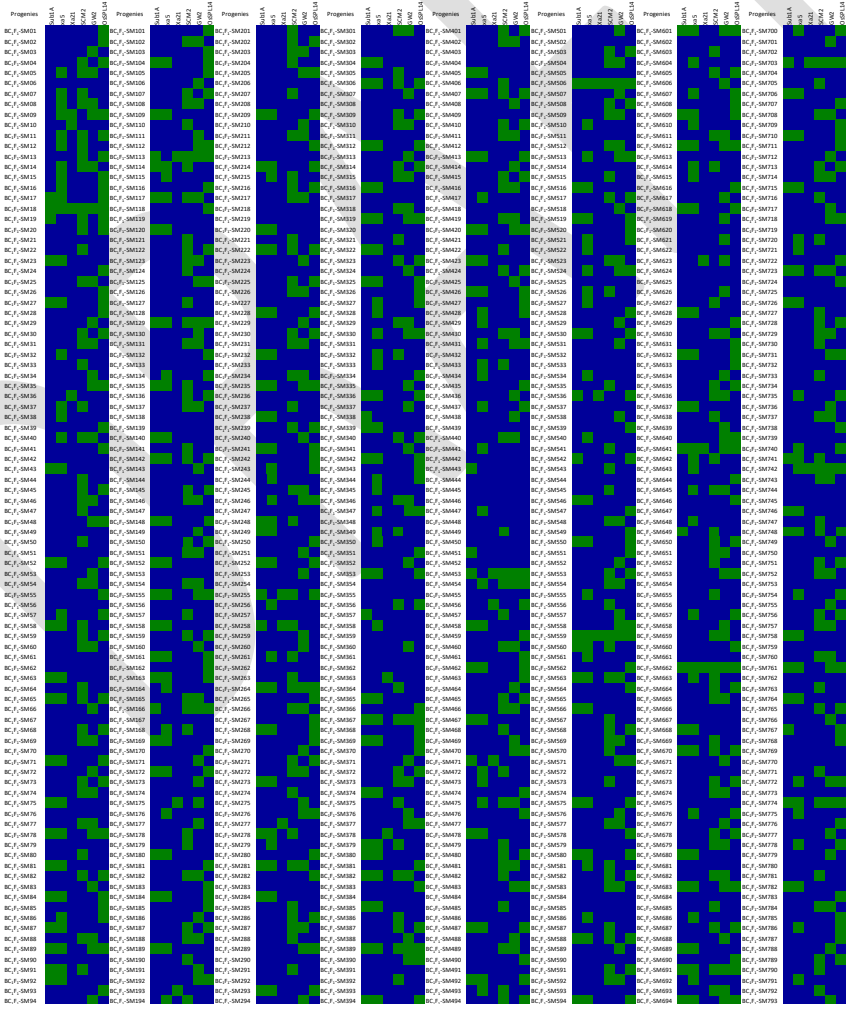


Fig 4. Banding analysis for submergence tolerance, bacterial blight resistance and yield components in BC₁F₁ derived lines for GW2, OsSPL14, SCM2, Sub1A, xa5 and Xa21 genes/QTLs. Blue and green represents absence and presence of a particular gene in a progeny. This figure provides six, five four three, two and one gene combinations genes/QTLs in the genotyping progenies.

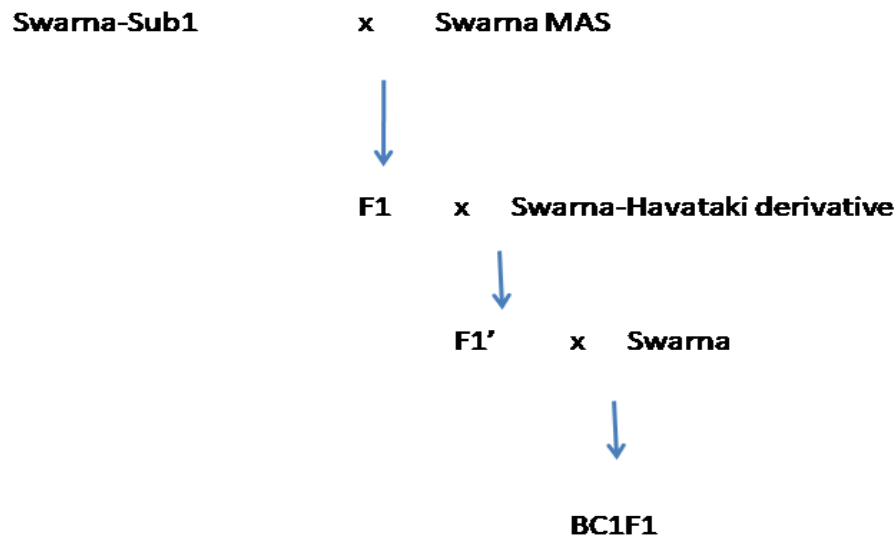


Fig 5. Schematic diagram for generation of backcross progenies for stacking of submergence tolerance, bacterial blight resistance and enhancing yield potential in rice variety, Swarna.

detected 241 plants positive for *xa5* gene and 125 plants positive for *Xa21* resistance gene individually (Fig. 2; Fig. 3).

The tightly linked molecular markers for some prominent traits have been identified and used for marker-assisted backcross breeding in rice including resistance to biotic stress bacterial blight resistance and abiotic stress submergence tolerance and additionally yield potential. Marker assisted gene pyramiding of key genes/QTLs has helped in tacking susceptibility for fore most diseases such as bacterial blight (Huang et al., 1997; Singh et al., 2001; Suh et al., 2011) and abiotic stress traits such as submergence tolerance (Neeraja et al., 2007; kharuddaula et al., 2011; Khanh et al., 2013; Divya et al., 2014) and yield potential.

In this present study, we observed many desirable genes individually but when it comes to combinations of genes with all 3 different characteristics, we found 6 plants out of total 796 which contains all the six genes/QTLs namely *GW2*, *OsSPL14*, *SCM2*, *Sub1A*, *xa5* and *Xa21* (Fig. 3). These are the most desirable progenies which will be used for further experiments. In the same experiment, we also found progenies with 5 gene combinations. Among these 5 gene combination progenies, seven plants (*BC₁F₁*-SM129, *BC₁F₁*-SM264, *BC₁F₁*-SM367, *BC₁F₁*-SM389, *BC₁F₁*-SM691, *BC₁F₁*-SM774 and *BC₁F₁*-SM796) showed the gene combination of *GW2*, *OsSPL14*, *SCM2*, *Sub1A*, *xa5* whereas two plants (*BC₁F₁*-SM113 and *BC₁F₁*-SM703) had the same set of genes, but instead of *xa5* they possessed *Xa21* (Fig 3; Fig 4). We also detected 30 progenies which showed 4 genes in different combinations. The genotyping results of progenies also exhibited 69 progenies having 3 gene combinations, 259 progenies having 2 gene combinations and 283 progenies having only a single gene (Fig. 3; Fig. 4). These progenies with desirable genes in different combinations can also be used further for different backcrossing programs. With the aid of gene introgression, this can lead to the development of submergence tolerant,

bacterial blight resistant and high yield potential cultivar in Swarna background that may be more popular than the parent, Swarna.

Materials and Methods

Parental lines Swarna-Sub1, Swarna MAS were raised in 7 days staggered for hybridization. Crossing was performed using Swarna-Sub1 as female parent. Next season, the true *F₁* plant was raised and crossed with Swarna- Havataki derivative to obtain *F₁'* seeds. True hybridity was again checked using *OsSPL14* gene specific marker and true *F₁'* plant was hybridized with recipient parent Swarna to produce *BC₁F₁* seeds.

Nursery of seven-ninety-six *BC₁F₁* generation plants of Swarna derived lines of Swarna derived plants for submergence tolerance, bacterial blight resistance and yield QTLs for were planted. Here, QTL referred to Quantitative Trait Locus is a section of the DNA that is associated with a specific phenotypic trait and is correlated to polygenic effects. The progenies were grown in big trays in RGA-cum-Phytotron facility chamber under controlled condition. Thirty days old seedlings were transplanted in the main field and grown with the following standard agricultural practices. The schematic representation of the parent and pedigree of plants is shown below

DNA Isolation

Leaves of 30 days-old seedlings were collected from 796 progenies to extract genomic DNA for molecular screening of yield QTLs, submergence tolerance and bacterial leaf blight resistance in derived *BC₁F₁* lines. Genomic DNA was extracted after crushing the sample in liquid nitrogen in microfuge tubes using Cetyltrimethyl ammonium bromide (CTAB) extraction buffer (100mmol/L MTris-HCl in pH-8, 20mmol/L Ethylene-

diamine-tetra-acetic acid (EDTA) pH-8, 1.3 mol/L NaCl, 2% CTAB) and chloroform-isoamyl alcohol followed by the RNase treatment and ethanol precipitation (Murray and Thompson, 1980). Agarose gel electrophoresis was used to estimate DNA concentration and each sample was then diluted to approximately 30ng/μl.

PCR amplification and visualization of markers

PCR amplification of rice the varieties and progenies was done with gene-specific primers for the QTLs SCM2, OsSPL14, GW2 and Sub1A, and closely linked molecular markers for xa5 and Xa21 (Table 1). DNA amplification reaction was performed in a volume of 20 μl containing 1.5mM Tris-HCl (pH 8.75), 50mM KCl, 2mM MgCl₂, 0.1% Triton X-100, 200mM each of Deoxy-adenosine-triphosphate (dATP), Deoxy-cytidine-triphosphate (dCTP), Deoxy-thymidine-triphosphate (dTTP), Deoxy-guanosine-triphosphate (dGTP), 4 pmol of each forward and reverse primers (Table 1), 1 unit of Taq polymerase and 30ng of genomic DNA. Amplification was performed in a programmable Thermal Cycler (Veriti; Applied Biosystems, Life Technologies, Singapore). The reaction mixture was first denatured for 4 min at 94°C and then subjected to 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55-58°C (Table 1) and 1min extension at 72°C, and then a final extension for 10 min at 72°C. Aliquots of 10 μl of DNA products from PCR amplification were loaded in 2.5% agarose gel containing 0.8 μg/ml ethidium bromide for electrophoresis in 1X Tris-Borate-EDTA (TBE) (pH 8.0). A 50 bp DNA ladder was used for the determination of the size of amplicons. The gel was run at 120V (2.5 V/cm) for 3 hours and photographed using a Gel-Doc System (SynGene). Molecular markers used for genotyping rice genotypes of the Sub1 gene, BB resistance genes and yield QTL cluster. The data were scored as 1/0(green/blue-Fig 3; Fig 4) for the presence/ absence of each target genes for primer-variety combination.

Conclusion

Submergence tolerance with bacterial leaf blight resistance and yield potential improvement of popular rice variety Swarna may be possible using component trait genes and QTLs viz., Sub1A, xa5, Xa21, GW2, OsSPL14 and SCM2. The presence of these genes was detected in the backcross progenies in BC1F1 generation. The presence of bands for all six genes was seen in only six progenies. These plants will be used for further selection and evaluation studies.

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